Phenomics of root system architecture: Measuring and analyzing root phenes

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Why study root system architecture?

For millennia, plant breeders have only had indirect information about the hidden root system. Now we can look at roots directly, and correlate root phenes (traits) to yield, nutrient uptake efficiency and drought resilience. These insights are invaluable for breeding improved crops.

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Root system architecture formation

A. Single root

B. Root system development

Formation of hydrophobic barriers in the endodermis

As the root develops, it creates hydrophobic structures in the endodermis layer. This development occurs in three stages:

1. Stage I - Formation of Casparian strip
   - Made of lignin
   - Blocks ions

2. Stage II - Formation of a suberin lamellae
   - Made of suberin
   - Blocks water

3. Stage III - Formation of a tertiary cell wall
   - Made of cellulose
   - Mechanical role

What does a root system look like?
All plants from a seed begin with a primary or tap root...

Root system formation is different in monocots and dicots


Root system formation is an iterative process

One primary root (PR, panel A) ↓
(Seminal roots, SR, panel B-C) ↓
Lateral roots (LR, panel C) ↓
(Crown and brace roots, CR & BR, panel C-D) ↓
More lateral roots, of higher order

Root system architecture is highly dynamic

Root system formation is different in monocots and dicots

Where are the soil resources, where are the roots and what are they doing?

A. The soil

Soil is composed of different sized particles, organic matter, water, and living organisms

Soil texture triangle

Particle size: Sand > Silt > Clay

Ions can diffuse from:

Solution to root (D1), particle surface to root (D2), exchange sites on particle (D3), or particle to solution (D4).

Ions can also travel with water to the root surface through the pores of the root (D5).

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Ions can also travel with water to the root surface through the pores of the root (D5).
Immobile nutrients bind to soil particles, so hard for roots to acquire. Depletion zones are small.

Mobile nutrients move in water, so easy for roots to acquire. Depletion zones are large.

Some plants have more lateral roots than others, why does it matter?

Many roots are beneficial for acquisition of immobile resources

Many roots are not beneficial for acquisition of mobile resources

Shallow-angled roots are useful for shallow resources like immobile phosphorus

Steep-angled roots are useful for deep resources like mobile water and nitrate
Where are the soil resources, where are the roots and what are they doing?

Water uptake by roots is a complex process.

Phenes are the basic units of phenotype and have a 1:1 conceptual mapping to genetic terms.

Phenes interact with one another to influence plant functions that contribute to performance and eventually yield.

Basic root phenes are really simple.
Phenomics uses phenotyping to quantify phenes of many plants and how these relate to plant performance.

How to describe root system architecture

B. Root system geometry

- Geometrical information
  - What you need: Position in space of every root segment
  - What you get: Depth, Width, Convex hull area, Length Profile

- Geometrical phenes can be used as proxy for other phenes
  - The length distribution profile (D\textsubscript{95}) is linked with the root angle

Identical width and depth, but very different root systems

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C. Root system morphology

- Morphological information
  - What you need: Tracing of a sample of the roots
  - What you get: Diameter, Length, Orientations

- Root systems can be seen as populations of roots

Morphological phenes can be used as proxy for other phenes

Similarly in small sample plants, trying to identify the correct proxy phene to acquire morphological phene...
Apical diameter is a proxy for root growth rate

Parent root apical diameter can be a proxy for lateral root apical diameter

How to describe root system architecture

D. Root system topology

Topological information

What you need
- Explicit relation between different root orders

What you get
- Lateral number
- Inter-lateral distance
- Insertion angle
- Length of Unbranched Apical Zone (LAUZ)

Extracting topological information can be hard for large root systems

Topological indexes

Altitude $|a|$ = length of longest path
Magnitude $|\mu|$ = sum of all external links
Topological index = slope of the regression between $a$ and $\mu$

Altitude of segment = the number of connections downstream the segment
Magnitude of segment = sum of all external links of the segment

How to describe root system architecture

E. Root system dynamics

What you need
- Time series images
- Follow the roots individually

What you get
- Growth rates
- Root emergence rates

Root elongation can also be linked to morphological/topological phenes such as the length of the apical unbranched zone (LAUZ)
3 How to describe root system architecture

F. Root System Markup Language

Any root system architecture can be stored into a Root System Markup Language format (RSML).

Root system architecture is complex and multidimensional.

Figure 2: visual representation of the RSML structure. A. Original image B. Graphical representation of the structure. Topology (primary root in red and lateral roots in green), geometry and properties are represented at different levels. C. Schematic representation of an RSML file structure. D. Representation of the coupling between the root geometry and its associated functions (here the diameter). Dotted lines represent data from the same point in a polyline.

How to acquire root architecture data

Usually a trade-off between the “naturalness” of the growing setup and its throughput.

Root excavations and trenching

Pros:
- 3D growth environment
- Soil conditions
- Constraints to growth
- Natural conditions
- Full lifespan of plant possible

Cons:
- Portion of root system only
- Destructive
- Time consuming

Pros:
- Soil condition
- Natural atmospheric conditions
- Full lifespan of plant possible

Cons:
- Only a portion of root system is visible

The RSML format is compatible with a wide range of tools. This enables:
- Interoperability
- Repeatability
- Meta analysis
- ...
Pros:
- No physical constraints to growth
- Artificial root environment
- 3D growth environment
- Full lifespan of plant possible
- Homogenous conditions
- Direct access to the roots for sampling

Cons:
- Limited oxygenation
- Artificial conditions
- Limited root system visible
- Segmentation and analysis difficult
- Very expensive

4. Hydroponic growth

Pros:
- No physical constraints to growth
- Artificial root environment
- 3D growth environment
- Full lifespan of plant possible
- Homogenous conditions
- Direct access to the roots for sampling

Cons:
- Limited root system visible
- Segmentation and analysis difficult
- Very expensive
As for the growing setups, there is usually a trade-off between the complexity of the images, the depth of the analysis, and its throughput.

Analyzing root images

Analyzing data using aggregation

The many individual properties of each individual root can be summed or averaged for the different root types to give aggregate properties of the entire root system.


Relating phenes to function using regression

Linear regression can be used to relate variation in root phenes to variation in plant mass, nutrient content, yield, or other measures of plant performance. This provides evidence for a direct relationship between root system phenotype and function.

The first 4 graphs show the relationship of 4 root phenes to shoot mass. Bottom panel shows multiple regression using all 4 phenes above to predict shoot mass more accurately.

Dimension reduction with Principal Component Analysis (PCA)

Multiple phenes can be reduced to a single phene aggregate based on ‘linear combinations.’ In this example, PC1 represents phenes influenced by node position and related to root size. PC2 are those phenes that are independent of node position.


Conclusions

Understanding how roots forage for soil resources is imperative for closing the yield gap (the gap between theoretical and actual yields) while the global population grows and climate changes. Therefore, acquiring root data quickly and accurately is an important challenge. This challenge is being met using various imaging approaches coupled to image analysis, borrowing from the fields of computer vision and machine learning. The phenomics of root system architecture will allow the integration of beneficial phene states in elite crop varieties to feed the world.